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Uudekall, Peep

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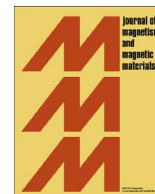
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# Atomic layer deposition of titanium oxide films on As-synthesized magnetic Ni particles: Magnetic and safety properties



Peep Uudeküll<sup>a,\*</sup>, Jekaterina Kozlova<sup>a</sup>, Hugo Mändar<sup>a</sup>, Joosep Link<sup>c</sup>, Mariliis Sihtmäe<sup>b</sup>, Sandra Käosaar<sup>b,d</sup>, Irina Blinova<sup>b</sup>, Kaja Kasemets<sup>b</sup>, Anne Kahru<sup>b</sup>, Raivo Stern<sup>c</sup>, Tanel Tättä<sup>a</sup>, Kaupo Kukli<sup>a,e</sup>, Aile Tamm<sup>a</sup>

<sup>a</sup> Institute of Physics, University of Tartu, W. Ostwaldi Str.1, 50411 Tartu, Estonia

<sup>b</sup> Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, 12618 Tallinn, Estonia

<sup>c</sup> Laboratory of Chemical Physics, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, 12618 Tallinn, Estonia

<sup>d</sup> Faculty of Chemical and Materials Technology, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia

<sup>e</sup> University of Helsinki, Department of Chemistry, P.O. Box 55, FI-00014 Helsinki, Finland

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## ABSTRACT

Spherical nickel particles with size in the range of 100–400 nm were synthesized by non-aqueous liquid phase benzyl alcohol method. Being developed for magnetically guided biomedical applications, the particles were coated by conformal and antimicrobial thin titanium oxide films by atomic layer deposition. The particles retained their size and crystal structure after the deposition of oxide films. The sensitivity of the coated particles to external magnetic fields was increased compared to that of the uncoated powder. Preliminary toxicological investigations on microbial cells and small aquatic crustaceans revealed non-toxic nature of the synthesized particles.

## 1. Introduction

During the past few decades, the use of fine magnetic particles in biomedical applications has gone through rapid growth. However, uniform size micro- and mesoscale particles have attracted far less attention than ultrafine particles as scientists are striving for new nano-applications. In one of the earliest works by Widder et al. [1] in late 1970s, mesoscale (nanometers to microns) magnetic particles were used for magnetic delivery of chemotherapy compounds to a specific site *in vivo*. In 1990, Miltenyi et al. [2] suggested a magnetic cell separation process in which a specially designed steel microfiber filled with fluorescently labeled magnetic microparticles was used in a separation column in order to isolate a specific cell population from a heterogeneous sample. A decade later, Mah et al. [3] were the first in the field of gene transfection using magnetic microparticles to deliver genes into cells by linking a viral vector to the particles.

The amount of research done has been growing ever since. The development of applications based on the most basic principle of magnetism, i.e. magnetic interaction at a distance, can revolutionize fields such as cancer and gene therapy, tissue engineering, developmental biology etc. For example, transition metal mesoparticles will be more used in a wide range of biomedical applications, such as magnetic

cell separation, drug targeting, magnetic fluid hyperthermia and other magnetism-based health applications [4].

Out of the array of transition metals, nickel is one of the most important magnetic materials with properties, that make it suitable for several previously mentioned applications [5,6]. However, several magnetic particles (including Ni) possess danger to living organisms not only due to toxic properties, but also due to other factors, such as particle aggregation [7]. These circumstances can possess catastrophic consequences, as clustered particles may block blood flow if applied *in vivo*. However, by coating the particles with biocompatible substances (e.g. TiO<sub>2</sub>), it is possible to increase the safety of the particles. This kind of safer-by-design approach has recently been shown by Gass et al. [8], who applied TiO<sub>2</sub> coating to various nanomaterials (CeO<sub>2</sub>, Fe<sub>2</sub>O<sub>3</sub>, ZnO, Ag) and observed significantly reduced toxicological effects compared to uncoated particles.

A surface functionalization method, whose potential is underused in the field of biomedicine, is atomic layer deposition (ALD). ALD belongs to a group of chemical vapour deposition (CVD) layer-by-layer techniques. It relies on self-limiting surface reactions of gases that are alternately introduced into and purged out of the reaction chamber, and consequently, it is possible to achieve conformal deposition with atomic precision on various nanostructured architectures with large

\* Corresponding author.

E-mail address: [peep.uudekull@ut.ee](mailto:peep.uudekull@ut.ee) (P. Uudeküll).

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aspect ratios [9,10]. Therefore, the alteration of the surface properties of the particles generally does not affect the bulk properties of the cores.

When targeting biomedical applications, one can propose using TiO<sub>2</sub> as the shell material due to its chemical and mechanical stability important for corrosion protection combined with photocatalytic properties for antimicrobial purpose [11,12].

Processes that can be used for fabricating Ni core mesoparticles include chemical reduction [13], sol–gel deposition [14], combustion reaction [6], etc. Among them, liquid phase methods have been widely applied because of their relative simplicity, flexibility and better control over the size of forming particles. The benzyl alcohol method is a widely applicable non-aqueous liquid phase approach for the synthesis of crystalline metal (oxide) structures in solution and at low temperatures – this solvothermal process includes the reaction of metal precursor with the benzyl alcohol as a liquid medium at temperatures in the range of 50–250 °C [15]. Owing to surfactant free fabrication, high crystallinity of the particles, dual role of the solvent, size controllability, etc., it has several advantages over other methods [16–18].

In the present study nickel mesoscale particles (100–400 nm) were synthesized using the benzyl alcohol method. The synthesized particles were subsequently coated with a thin TiO<sub>2</sub> layer by ALD. For ALD of TiO<sub>2</sub> on Ni particles, a well-known and earlier described reliable ALD process based on the application of titanium tetrachloride and water [19,20] was exploited. The size, morphology, magnetic properties and the toxicity of the coated and uncoated Ni mesoparticles to bacteria *Vibrio fischeri*, yeast *Saccharomyces cerevisiae* and freshwater crustacean *Thamnocephalus platyurus* were evaluated. The magnetic behavior of the particles was evaluated by vibrating sample magnetometer in order to explore the possibility to rapidly magnetize and demagnetize the metal sensing cores as well as the possible promotional or destructive effects of the oxide coatings on the metal.

## 2. Experimental details

### 2.1. Synthesis of particles

1.0 g of nickel(II)acetylacetonate (Sigma-Aldrich, 95%), was added to 20 mL of benzyl alcohol (Sigma-Aldrich, 99%) in a 100 mL round bottom flask, which was sealed and placed on a magnetic stirrer for 24 h. After obtaining a homogeneous solution, the mixture was heated on an oil bath at 180 °C for 24 h. The resulting black precipitate was filtered and repeatedly rinsed in pure acetone, in order to remove the chemicals adsorbed on the surface of the particles. The precipitate was dried in air at room temperature (25 °C), collected in the form of powder, and divided into two sets of samples. One of them was coated with TiO<sub>2</sub> film by ALD, after which both samples were characterized as described in 2.3.

### 2.2. Coating the particles via atomic layer deposition

The TiO<sub>2</sub> film on nickel powder was deposited in a flow-type in-house built hot-wall ALD reactor [20] using TiCl<sub>4</sub> (Sigma-Aldrich, 99.99%) and H<sub>2</sub>O as precursors. N<sub>2</sub> was used as a carrier gas while the reactor pressure ranged from 195 to 260 Pa. At first, thin amorphous nucleation layers of TiO<sub>2</sub> were grown by applying 10 ALD cycles at 150 °C followed by 300 ALD cycles at 300 °C. The cycle times were 30–30–0–0 s at 150 °C and 10–20–2–10 s at 300 °C for the TiCl<sub>4</sub> pulse, first purge, H<sub>2</sub>O pulse and the second purge period, respectively. The two-temperature process was applied in order to start the deposition at low temperature, i.e. using, presumably, a less destructive growth mode, which can also provide higher nucleation density due to the preferred growth of amorphous, disordered material. The deposition of TiO<sub>2</sub> was then completed by the growth at higher temperature providing more intense crystal growth and higher chemical purity [21].

### 2.3. Characterization

The surface morphology and thicknesses of TiO<sub>2</sub> films were evaluated by high-resolution scanning electron microscope (HRSEM) Helios NanoLab 600 (FEI) equipped with focused ion beam (FIB) module. The film thicknesses were determined from SEM images taken from sample cross-sections prepared by FIB or with spectroscopic ellipsometry (SE) on reference planar substrates. Compositions of coated and uncoated particles were measured by an energy dispersive X-ray spectrometer (EDS) INCA Energy 350 (Oxford Instruments). Structural characterization was performed by X-ray diffraction (XRD) analysis (Rigaku SmartLab), using CuK $\alpha$  radiation with diffraction angle (2 $\theta$ ) ranging from 15°–80°, and by Raman spectroscopy using a Renishaw *in via* micro-Raman spectrometer with the incident argon-ion laser beam wavelength of 514 nm and spectral resolution of 1.5 cm<sup>−1</sup>. The magnetic measurements were performed on the 14 T vibrating sample magnetometer – physical property measurement system (VSM-PPMS, Quantum Design). Hysteresis measurements were performed by scanning the magnetic field from  $-1.6 \times 10^6$  to  $+1.6 \times 10^6$  A/m at room temperature.

### 2.4. Toxicity testing

#### 2.4.1. Preparation of particles suspensions

Stock solution of uncoated and TiO<sub>2</sub>-coated Ni particles (5 g Ni/L) were prepared in deionized (DI) water (pH 5.6  $\pm$  0.1) (Milli-Q, Millipore) and the suspensions were homogenized using ultrasonic probe (Branson Digital Sonifier, USA) for 4 min at 40 W immediately after suspension preparation. Soluble nickel salt, NiCl<sub>2</sub>·6H<sub>2</sub>O (Merck) (5 g Ni/L in DI), was used as the ionic control for Ni particles and CuSO<sub>4</sub> (Alfa Aesar) (5 g Cu/L in DI) was used as the standard positive control for the selected toxicity tests.

#### 2.4.2. Toxicity evaluation using naturally luminescent bacteria *Vibrio fischeri*

The bioluminescence inhibition assay with luminescent bacteria *Vibrio fischeri* was used for toxicity evaluation of coated and uncoated Ni-particles. In this assay, the luminescence of the bacteria decreases in concentration-dependent manner upon exposure to toxic chemicals [22]. The 30-min kinetic bioluminescence inhibition assay (a Flash-test) was performed as described in Mortimer *et al.* following the standard protocol (ISO 21338, 2010) [23,24]. The assay was performed at room temperature ( $\sim$ 20 °C) on 96-well microplates using a Microplate Luminometer Orion II (Berthold Detection Systems, Pforzheim, Germany), controlled by Simplicity Version 4.2 Software. Reconstituted *V. fischeri* Reagent (Aboatox, Turku, Finland) was used as the test bacteria suspension and 2% NaCl as a diluent and as a negative control. Each test was performed in 5–7 replicates using exponential dilutions. Ni compounds were tested at nominal concentration range of 0.075–500 mg Ni/L.

The test results were expressed as EC<sub>50</sub> values (the nominal concentration of a compound reducing the bioluminescence by 50%) determined from the concentration-effect curves by the application of the log-normal model using REGTOX software for Microtox Excel™ (MS Excel macro REGTOX EV7.0.5.xls).

#### 2.4.3. Toxicity evaluation using yeast *Saccharomyces cerevisiae*

A 'spot' assay format described in Suppi *et al.* was used [25]. In this test yeast cells were exposed to different concentrations of the chemical of interest in DI water for 24 h and after that the colony-forming ability of the exposed and unexposed (control) cells on toxicant-free agarized growth medium was evaluated. As a result, a minimum biocidal concentration (MBC) of the chemical was determined.

*S. cerevisiae* BY4741 (EUROSCARF, Institute of Microbiology, University of Frankfurt, Germany) was used as a test organism. Briefly, 75  $\mu$ L of yeast cells in DI water ( $\sim 10^7$  viable cells/mL) were

exposed 1:1 to the test chemicals on 96-well microplates in DI water (total volume 150  $\mu\text{L}$  per well). DI water without test chemicals inoculated with the yeast cells served as the control. Ni compounds were tested from 0.01–1000 mg Ni/L (ten-fold dilutions). Test plates were incubated at 25 °C for 24 h without shaking in the dark. After 24-h exposure, 5  $\mu\text{L}$  of test culture from each microplate well was transferred as a spot onto toxicant-free yeast extract-peptone-dextrose (YPD) agar plates and incubated for 72 h at 30 °C. Minimum biocidal concentration (MBC) of the tested Ni compounds and  $\text{CuSO}_4$  (a positive control) was determined as the lowest tested nominal concentration which completely inhibited the outgrowth of the exposed cells, i.e. formation of visible colonies on toxicant-free agar medium.

#### 2.4.4. Toxicity evaluation using freshwater crustaceans *Thamnocephalus platyurus*

*T. platyurus* was used as a test organism and the test was performed as described in Standard Operational Procedures of Thamnotoxkit F™ (1995) [26]. Briefly, *T. platyurus* larvae obtained by the hatching of cysts were exposed to different concentrations of chemical of interest for 24 h at 25 °C in the dark and the mortality was used as the toxicity endpoint. The tests were performed in triplicate. The test results were expressed as  $\text{LC}_{50}$  values (the nominal concentration of a compound causing the death of 50% of the studied organisms) and were calculated using MS Excel macro REGTOX software (see above).

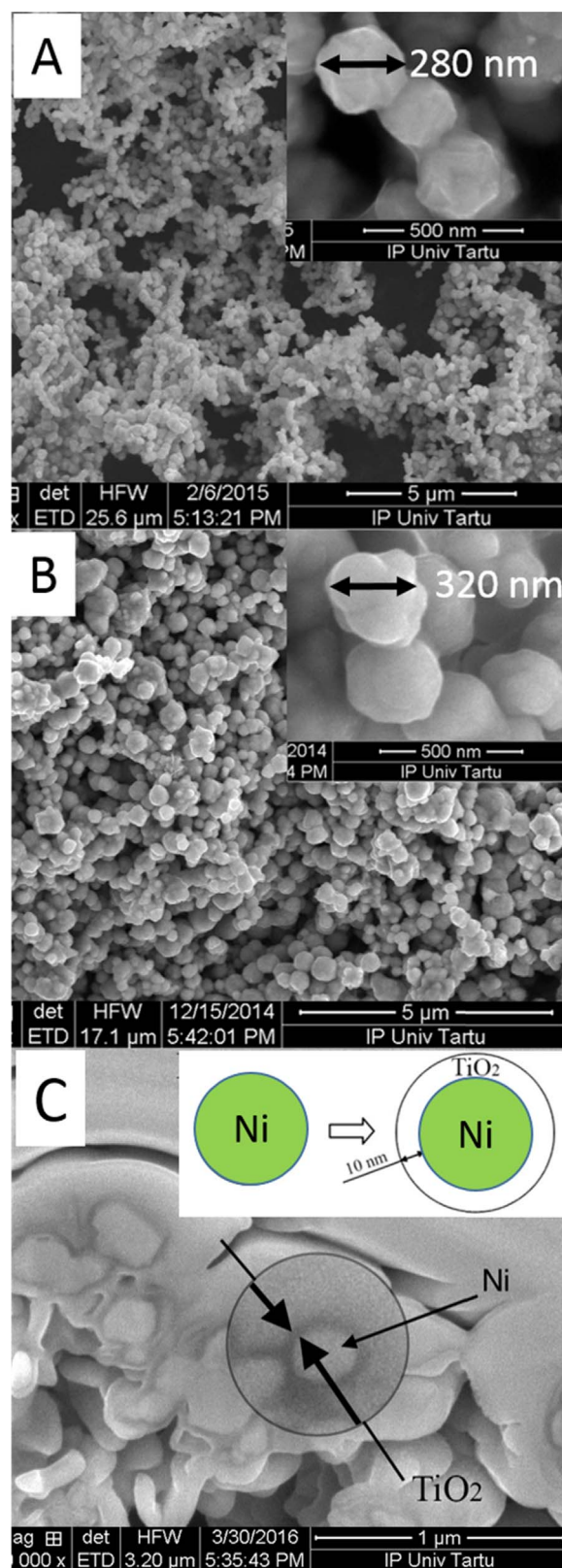
### 3. Results and discussion

Fig. 1 represents the SEM images of the uncoated and coated Ni particles and shows that both of the particles were almost spherical. It has been observed earlier that the benzyl alcohol based method provides spherical morphology of the particles [27]. Herewith the size of the particles synthesized in our study varied from 100 to 400 nm and the average size of the particle was close to 300 nm (Fig. 1A, inset). The particles appeared to be somewhat agglomerated, probably in order to minimize the total surface energy of the system [28], driven by their large surface-to-volume ratio and strong magnetic forces. The size and shape of  $\text{TiO}_2$ -coated Ni particles (Fig. 1B) were practically identical to their uncoated counterparts (Fig. 1A), as ALD enabled uniform deposition of 10 nm  $\text{TiO}_2$  layer (Fig. 1C). One can see that the  $\text{TiO}_2$ , distinct as the thin dark layer between conducting Ni particles and Pt coating, has uniformly covered the particles in the FIB-SEM image. The layer grown by ALD added about 20 nm to the total diameter of the particles.

Composition analysis of the Ni particles did not show considerable effect of oxygen on the composition (surface) of the particles, i.e. the oxidation of Ni (Table 1). The EDS analysis carried out on the particles coated with the  $\text{TiO}_2$  film revealed 3.7 wt% of residual chlorine. The results obtained demonstrated that, although the substrate temperature was low and the aspect ratio for the powder as the substrate was high, chlorine was quite efficiently removed from the film surface during the deposition process.

XRD patterns of the coated and uncoated Ni samples are presented in Fig. 2. Both patterns show three strong reflections at  $2\theta$  values of 44.45, 51.8 and 76.36° which can be indexed as 111, 200 and 220 of cubic (fcc) nickel (JCPDS card 4–0850). XRD crystallite size of Ni particles was 29 nm calculated from the integral width of the reflection 111 using the Scherrer formula. This crystallite size, compared to particle size of 300 nm (from SEM), indicates that Ni particles were mainly polycrystalline. The thin titanium dioxide coating on powder particles in sample (b) was recognized and identified by a weak and broad (XRD crystallite size 7 nm) reflection at 25.2° matching with the strongest reflection 101 of anatase (JCPDS card 65–5714).

In the Raman spectra taken from the uncoated Ni particles the peak at 1080  $\text{cm}^{-1}$  appeared, typical for Ni nanoparticles (Fig. 3) and after the growth of  $\text{TiO}_2$  film on the particles, anatase was recognized with



**Fig. 1.** SEM image of the uncoated (A) and  $\text{TiO}_2$ -coated (B) Ni particles and the SEM-FIB image of  $\text{TiO}_2$ -coated Ni particles (Fig. C). The particles (Fig. C) were coated with platinum (conductive material on isolator) to get better resolution on SEM image of thin  $\text{TiO}_2$  films on coated Ni particles.

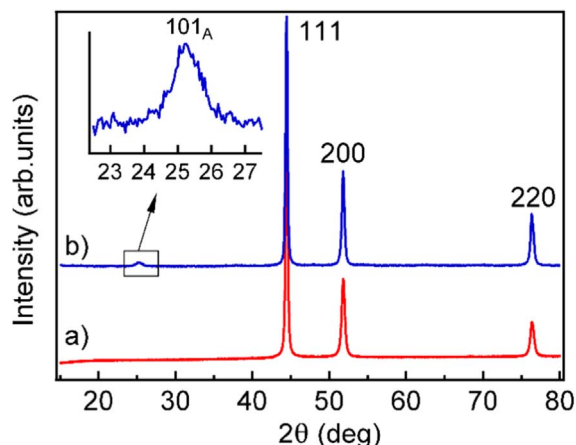
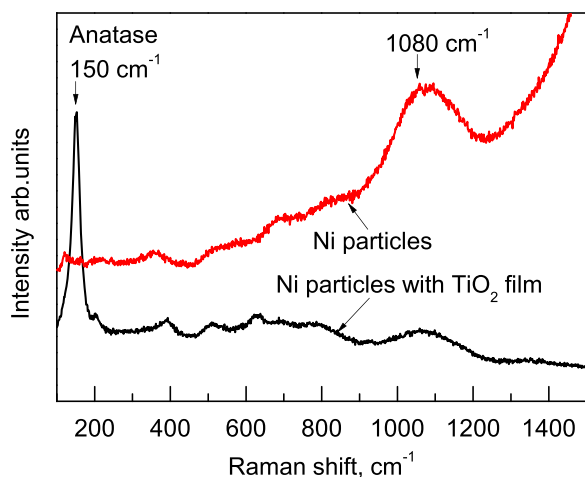
the characteristic peak at 144  $\text{cm}^{-1}$  [29].

It has been stated that nickel nanoparticles, in particular Ni nanowires, are not Raman active [30] and the Raman studies have



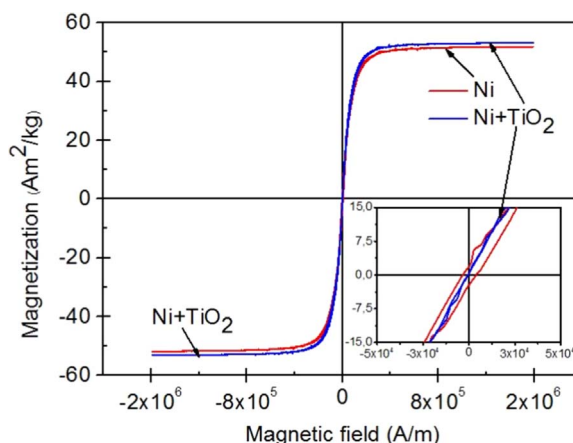
**Table 1**The EDX analysis of Ni particles (uncoated) and TiO<sub>2</sub>-coated Ni particles.

Element	C	O	Ni	Ti	Cl	Total
Ni	3,4%	0,7%	95,9%	0%	0%	100%
Ni + TiO <sub>2</sub>	2,2%	6,7%	78,4%	9,0%	3,7%	100%

**Fig. 2.** X-ray diffraction patterns of uncoated a) and TiO<sub>2</sub>-coated b) Ni samples. Miller indices of corresponding lattice planes are shown at peak top position.**Fig. 3.** The strongest reflection of anatase also detected is magnified in the inset (A). Room-temperature micro-Raman spectra of Ni particles and coated ones with TiO<sub>2</sub> films in Anatase phase (B). Raman peaks recognized are denoted by labels (B).

indeed mostly been carried out either on oxidized [31,32], or functionalized Ni, e.g. nickel-carbon core-shell composites [33]. Thus, decided on the basis of the Raman spectra in the present study, one can also not exclude partial oxidation of nickel particles.

The magnetization curves (Fig. 4) of the samples clearly demonstrate saturation regime in the magnetization-field curves. The curves are also characterized by rather weak coercive forces, and, consequently, narrow hysteresis loops. This is characteristic of soft magnetic materials and may be regarded as a prospective property for a magnetic sensor material that is to be quickly magnetized and demagnetized. The saturation magnetization  $M_s$  of coated and uncoated Ni particles measured at room temperature was 51 and 53 A × m<sup>2</sup>/kg respectively, which indicated that the deposition of thin TiO<sub>2</sub> film has increased the  $M_s$  by 4%. For reference, the  $M_s$  of bulk nickel is 55 A × m<sup>2</sup>/kg [34]. For further comparison, for Ni nanocrystals embedded in an alumina matrix [35], the  $M_s$  measured has been slightly higher that 67 A × m<sup>2</sup>/kg even though the particles were smaller by an order of magnitude, compared to those synthesized in the present study. However, the

**Fig. 4.** Magnetization curve of the nickel compound (red) and its ALD coated counterpart (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

coercivity ( $H_c$ ) was nearly immeasurable. In the present study,  $H_c$  for the TiO<sub>2</sub>-coated Ni was only 238 A/m while the uncoated counterpart possessed  $H_c$  of 3580 A/m. So it can be stated that the TiO<sub>2</sub>-coated Ni particles could demonstrate better magnetic sensor properties than the uncoated particles.

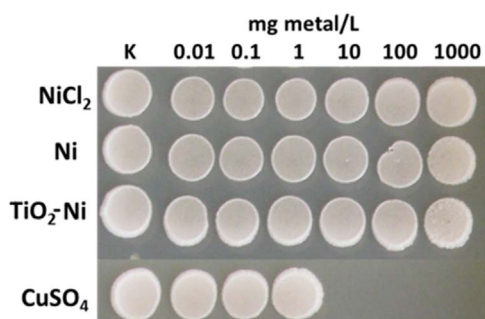
All substances on the European market manufactured or imported in quantities of 1 t or more per year have to be evaluated for their hazardous effects to humans and environment. This is in accordance with the European Union (EU) chemical safety regulations on Registration, Evaluation, Authorization and Restriction of Chemicals (REACH; EC, 2006) [36], and Classification, Labeling and Packaging (CLP) of substances and mixtures (EC, 2008) [37]. For that a number of standardised toxicity tests are developed and have to be applied, e.g. the basic ecotoxicological information requirements include short-term toxicity testing on invertebrates (preferred species *Daphnia*; OECD 202, 2004) [38] and growth inhibition study on aquatic plants (algae preferred; OECD 201, 2011) [39]. The preliminary toxicity screening on (eco)toxicological effects of uncoated and TiO<sub>2</sub>-coated Ni particles were conducted with two different groups of unicellular organisms, bacteria (a prokaryotic organisms) and yeast (a eukaryotic organism). In addition, a particle-ingesting aquatic crustacean was selected as a representative consumer at the food-web level (Table 2). The results of toxicity testing were expressed as EC<sub>50</sub>/LC<sub>50</sub>/MBC values based on nominal exposure concentrations. For the hazard ranking of the tested particles following criteria were applied: EC<sub>50</sub>/LC<sub>50</sub>/MBC value < 1 mg/l very toxic; 1–10 mg/l = toxic; 10–100 mg/l = harmful; > 100 mg/L = not classified/not harmful [40].

Since bacteria are ubiquitous organisms in soil and water, being important link in the terrestrial and aquatic food-webs as decomposers, *Vibrio fischeri* bioluminescence inhibition assay was chosen. No inhibition of luminescence by Ni uncoated and TiO<sub>2</sub>-coated particles was observed even at 500 mg Ni/L, i.e. Ni particles could be considered as non-hazardous according to the used bacterial test. The latter was also supported by the parallel analysis of the toxicity of respective soluble Ni-salt; the 30-min EC<sub>50</sub> value for NiCl<sub>2</sub> was 408.7 mg Ni/L (Table 2). The 30-min EC<sub>50</sub> values at 15 °C (optimal temperature for bacteria *Vibrio fischeri*) obtained by other authors for NiCl<sub>2</sub> (as for Ni) were in the range of 42.2–208.7 mg/L [41,42] and are 2–10 fold lower compared to the results from the current study at 20 °C (as 15 °C is not compatible with the conventional plate luminometers). However, this difference can be explained by the different testing conditions used (e.g., 15 °C vs 20 °C).

*Saccharomyces cerevisiae* viability (colony-forming-ability) inhibition assay is not conventional standard test for the environmental hazard evaluation. In this study the yeast cells were studied as a model for the eukaryotic cell as *S. cerevisiae* cells have quite similar cellular

**Table 2**Toxicity of uncoated and TiO<sub>2</sub>-coated Ni particles to bacteria *Vibrio fischeri*, yeast *Saccharomyces cerevisiae* BY4741 and crustaceans *Thamnocephalus platyurus*.

Test species	Bacterium <i>V. fischeri</i>	Yeast <i>S. cerevisiae</i>	Freshwater crustacean <i>T. platyurus</i>
Characterization of the test species	Prokaryotic unicellular organisms, a priori particle-proof	Eukaryotic unicellular organisms. particles endocytosis activity	Multicellular invertebrate; particle-ingesting organisms
Toxicity (mg metal/L) and toxicity endpoint)	30-min EC <sub>50</sub> <sup>a</sup> [mg/L] (inhibition of luminescence)	24-h MBC <sup>b</sup> [mg/L] (colony forming ability)	24-h LC <sub>50</sub> <sup>c</sup> [mg/L] (mortality)
<b>CHEMICALS TESTED</b>			
TiO <sub>2</sub> -coated Ni particles	> 500	> 1000	521
Uncoated Ni particles	> 500	> 1000	662
NiCl <sub>2</sub> ×6H <sub>2</sub> O (ionic control)	409	> 1000	5.50 <sup>d</sup>
CuSO <sub>4</sub> (positive control for the test validation)	0.16	10.0	0.012

<sup>a</sup> EC<sub>50</sub> – half-effective concentration;<sup>b</sup> MBC – minimum biocidal concentration – the lowest tested concentration that completely inhibited the visible growth/colony formation of yeast on the agarized growth medium after 24-h exposure to the tested compounds.<sup>c</sup> LC<sub>50</sub> – half-lethal concentration. All concentrations are nominal. Tentative hazard ranking: ≤1 mg/L, very toxic; > 1–10 mg/L, toxic; > 100 mg/L, not classified/not harmful [35].**Fig. 5.** Colonies (visible as ‘spots’) of yeast *Saccharomyces cerevisiae* on agarised growth medium. Ni-compounds showed no effect of colony forming potential even at 1000 mg Ni/L. Absence of growth (spots) in case of Cu-ions (a positive control) at exposure concentrations higher than 1 mg Cu/L indicates toxic effects.

structure and particles endocytosis ability as mammalian cells and also as a fungal model as the yeast cells different from mammalian cells have a thick outer mannan- $\beta$ -glucan-chitin cell wall [43,44].

We have previously demonstrated that algae and crustaceans are the most sensitive and thus probably the most vulnerable organism groups in aquatic exposure to nanomaterials [40]. According to the data obtained in the present study, both uncoated and TiO<sub>2</sub>-coated Ni particles, were not toxic to *T. platyurus* (24-h EC<sub>50</sub> > 500 mg Ni/L). However, soluble Ni-salt (NiCl<sub>2</sub>) proved to be harmful to *T. platyurus* (24-h EC<sub>50</sub>=5.5 mg Ni/L) (Table 2) indicating that the Ni-particles may cause toxicity to crustaceans if the conditions support their solubilisation (i.e. shedding of Ni-ions).

Results showed that the uncoated and TiO<sub>2</sub>-coated Ni particles and Ni-ions did not affect adversely the viability (colony forming ability) of the yeast *S. cerevisiae* cells up to 1000 mg Ni/L (MBC > 1000 mg Ni/L) (Table 2, Fig. 5).

Summing up, short-term toxicity screening showed that both uncoated as well TiO<sub>2</sub>-coated Ni-particles can be classified as nontoxic substances to bacteria, yeast and crustaceans.

#### 4. Summary

Nickel particles were synthesized via benzyl alcohol method. The reaction resulted in spherical shape particles, predominantly in the size range of 100–400 nm. The surface of the particles was successfully coated with 10 nm thick TiO<sub>2</sub> film in anatase phase by ALD method. Magnetic measurements indicated weak hysteresis in the case of uncoated Ni particles compared to the even smaller coercivity in their coated counterpart. Saturation magnetization can be recorded in both coated and uncoated cases. Uncoated and TiO<sub>2</sub>-coated Ni particles proved to be not harmful according to the bioassays used (bacteria, yeasts, crustaceans) pointing to the non-toxic nature of the synthesized particles and indicating a promising base for future applications. Since

the nickel particles coated with thin titanium oxide layers responded to the variations of external magnetic fields, rapidly magnetizing and demagnetizing, as measured and reported, one can consider certain potential for the application of the materials studied in the biocompatible sensorics. Therefore, it is necessary to further analyze biocompatibility (i.e. to conduct assays on mammalian cells *in vitro*), surface functionalization and magnetic properties, which are the biggest challenges in complex biological environments.

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